cartilage destruction in vitro. These effects are not mediated by the inhibition of histone deacetylases or the induction of the Nrf2 transcription factor but likely act via NFκB signalling. We are currently pursuing these findings in vivo.

275

HDAC1, 2 AND 7 AS MODULATORS OF PROTEOGLYCAN PRODUCTION: THE EFFECTS OF SEPARATE AND SIMULTANEOUS KNOCKDOWN

R. Bakker [1], J.E. Bekkers [1], A.H. Kragten [1], D.B. Saris [1,2], W.J. Dhert [1,3], L.B. Creemers [1], L.A. Vonk [1]. [1] *UMC Utrecht, Utrecht, Netherlands;* [2] *MIRA Inst., Univ. of Twente, Enschede, Netherlands;* [3] *Vet. Med., Univ. of Utrecht, Utrecht, Netherlands*

Purpose: Histone deacetylases (HDACs) regulate gene expression by histone modification. HDAC1, 2 and 7 expression is increased in OA patients and they are associated with downregulation of several chondrogenic genes and increased matrix metalloproteinase-13 expression. Therefore, this study investigated histone deacetylase knockdown as a possible target to modulate cartilage metabolism.
Methods: OA chondrocytes were obtained from patients undergoing knee arthroplasty. At passage 2, OA chondrocytes were transfected with siRNA against HDAC1, 2, and 7 or a mock, or they were treated with the isotype-selective HDAC inhibitor mocetinostat. Chondrocytes were reverse-transfected during seeding at high density (1.26*106 cells per cm2) on collagen-coated culture inserts in a 96-wells transwell system. Real-time PCR was performed to examine gene expression levels of HDAC1, 2 and 7, aggrecan (ACAN), type I collagen (COL1A1), type II collagen (COL2A1) and matrix metallopeptidase 13 (MMP13). HDAC1, 2 and 7 were analyzed at the protein level by Western blot. Glycosaminoglycan (GAG) and DNA content were determined using a DMMB and Picogreen assay, respectively.
Results: Expression levels of the targeted HDAC were decreased by RNAi. No differences were observed in GAG and DNA content between the chondrocytes transfected with mock and HDAC1, 2 or 7-directed siRNA. Transfection with HDAC1, 2 or 7 siRNA showed no effects on gene expression levels of ACAN, COL1A1, COL2A1 and MMP13. Knockdown of HDAC1 resulted in significantly higher HDAC2 gene expression levels and a trend towards higher HDAC7 gene expression levels. Knockdown of HDAC2 increased HDAC1 and HDAC7 gene expression levels and knockdown of HDAC7 resulted in increased HDAC1 and HDAC2 gene expression levels. Transfection with HDAC1, 2, and 7 siRNA together, and treatment with the isotype-selective HDAC inhibitor mocetinostat increased the amount of GAG produced by the cells.
Conclusions: Specific knockdown of HDAC 1, 2 or 7 did not improve the GAG production by OA chondrocytes, most likely due to the compensatory upregulation of the other HDAC subtypes. Hence, HDAC 1, 2 and 7 seem to have overlapping functions in modulating the GAG production of chondrocytes. Knockdown of HDAC1, 2 and 7 together did increase GAG production. This suggests that modulation of multiple HDACs, is necessary for gene-based treatment of OA.

276

ACTIVATION OF NF-κB/P65 INITIATES CHONDROGENIC DIFFERENTIATION OF CHONDROPROGENITOR CELLS

M.J. Caron [1], P.J. Emans [1], D.A. Surtel [1], A. Cremers [1], J.-W. Voncken [2], T.J. Welting [1], L.W. van Rhijn [1]. [1] *Dept. Orthopaedic Surgery, Maastricht Univ. Med. Ctr., Maastricht, Netherlands;* [2] *Dept. Molecular Genetics, Maastricht Univ. Med. Ctr., Maastricht, Netherlands*

Purpose: For the purpose of cartilage and bone regenerative techniques, the initiation of chondrogenic differentiation from progenitor cells is an important and precarious step that must be well-guided. NF-κB/p65 is reported to be involved in regulation of chondrogenic differentiation. However, whether it is involved in the initiation of chondrogenesis and its relation to key chondrogenic transcription factor Sox9 and is poorly understood. We hypothesized that the early onset of chondrogenic differentiation is initiated by transient NF-κB/p65 signaling.
Methods: The role of NF-κB/p65 in early chondrogenesis was investigated in different chondrogenic models: ATDC5 cells, hBMSCs, chicken periosteal explants and mouse growth plates. NF-κB/p65 activation was manipulated using pharmacological inhibitors, RNAi and activating agents. Gene expression and protein expression analyses, and (immuno)histochemical stainings were employed to determine the role of NF-κB/p65 in early chondrogenesis.
Results: All of the employed chondrogenic models showed that early chondrogenic differentiation correlates with transient activation of NF-κB/p65 and expression of its transcriptional targets (e.g. COX-2, TNFα, iNOS). This early NF-κB/p65-mediated signaling leads to early transient expression of Sox9 and determines the subsequent chondrogenic outcome (Col2a1 and Col10a1). Subtle stimulation of NF-κB/p65 activity resulted in increased expression of cartilaginous markers in both our *in vitro* as well as periosteal explant cultures, even in the absence of known chondrogenic growth factors. In addition, early NF-κB/p65 activity and Sox9 expression were enhanced by BMP-2, providing a possible explanation for BMP-2 driven chondrogenic differentiation.
Conclusions: The herein presented data demonstrate that NF-κB/p65 signaling, as well as its intensity and timing, represents one of the transcriptional regulatory mechanisms of the chondrogenic developmental program of chondroprogenitor cells. Importantly, this concept may be used as a molecular tool to modulate chondrogenic differentiation and thereby improve the success of cartilage regenerative techniques.

277

COG5 INHIBITION INDUCES GLYCOSYLATION DEFECTS AFFECTING CHONDROGENESIS AND INTERFERING WITH WNT, BUT NOT BMP SIGNALING

F. Cailotto, F.P. Luyten, R.J. Lories. *Katholieke Univ.it Leuven, Leuven, Belgium*

Purpose: Osteoarthritis (OA) is a highly disabling pathology of the bone-cartilage unit, to which both patient-dependent (weight excess, trauma…) and genetic factors contribute. Recently, a locus on Chromosome 7 (7q22) was linked to knee and hand OA by genome wide association study. In this locus, the Conserved oligomeric Golgi complex subunit (COG) 5 gene encodes a component of Golgi apparatus. Mutations in COG5 are associated with mild congenital glycosylation disorders, and COG5 expression is higher in 3D than in monolayer cultures of articular chondrocytes. As aspects of endochondral bone formation are recapitulated in OA (loss of stable phenotype, hypertrophy), we investigated the role of COG5 in chondrogenesis.
Methods: We used the ATDC5 chondrogenic cell line. Three independent stable clonal colonies of each, control (empty vector) and COG5 knocked-down cells (COG5-), were selected over 7 days (D) using puromycin. Clones were cultured as high density micromasses (2x105 cells/10 μl) to mimic the condensation step of developing cartilage in vivo. Chondrogenesis was induced by culturing cells for 14 D in DMEM/F12 + 5% FBS, supplemented with ITS [insulin (10 μg/ml), transferrin (10 μg/ml) and sodium selenite (30 nM)]. On D14, cells were switched to mineralization medium (αMEM + 5% FBS, supplemented with ITS) to investigate the mineralization phase of chondrogenesis. mRNA expression of typical chondrogenesis markers (Aggrecan (Agg), type II (Col2a1) and X (Col10a1)) collagens were assessed by quantitative RT-PCR. Likely, we assessed mRNA expression of Wnts (-4, -5a, -5b, -11) and BMPs (-2, -4, -6, -7). Quantification of Alcian Blue, Safranin O, Sirius red and Alizarin red staining were used to evaluate proteoglycans, collagens and mineralized content respectively. Western blot was used to check the glycosylation status of Wnts, and the activation status of Smad 1/5/8, typically increased in the proliferative phase of chondrogenesis. Cell viability was checked with Live/Dead® assay.
Results: During the early proliferation (D1-7) and late proliferation/hypertrophy phase (D7-14), Col2a1 and Agg mRNA were strongly increased, but to a lesser extent in COG5-. Safranin O and alcian blue staining were also less upregulated in COG5-. Pro-chondrogenic BMP-4 increased, and was surprisingly significantly higher in COG5-. This was consistent with a higher activation of Smad 1/5/8 in COG5-. Wnt-4, -5a and -5b mRNA increases were similar in control and COG5- cells. BMP-6 and Col10a1 mRNA were increased at D14, but significantly lower in COG5-. A strong Wnt-11 mRNA increase was observed at D7-14 in controls, but not in COG5-. Moreover, analysis of Wnt-5a in culture supernatants revealed a glycosylation defect in COG5-. Sirius red increased over time, and was significantly higher in controls. However, no difference was detected in cell viability. During mineralization phase (D14 to D21), BMP-2 and -7 mRNA